

GWAS in a Korean Soybean Core Collection Reveals Genetic Dissociation between Dehydration Response and Oxidative Stress under Drought

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Abstract

Background

Drought stress is one of the major environmental factors limiting soybean (*Glycine max* (L.) Merr.) productivity and is an increasing concern under changing climate conditions. Drought tolerance is a complex trait involving multiple physiological processes, including the maintenance of plant water status and the regulation of oxidative stress.

Results

In this study, drought responses were evaluated in a Korean soybean core collection using three physiological traits: wilting score, leaf moisture content (LMC), and H₂O₂ content. Wilting score and LMC shared a major association locus on chromosome 10, whereas H₂O₂ content showed only weak correlations with the dehydration-related traits and was associated with a distinct locus on chromosome 13. *Glyma.10G019000* and *Glyma.13G183500* were identified as candidate genes potentially involved in plant water-status regulation and ROS homeostasis, respectively, under drought stress.

Conclusion

These findings suggest that dehydration-related traits and ROS regulation involve distinct genetic components of the broader drought response in soybean. The candidate genes and favorable haplotypes identified in this study provide valuable genetic resources for understanding the genetic basis of drought responses and for developing molecular breeding strategies to improve drought tolerance in soybean.

Background

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important crops, providing a major source of plant protein and vegetable oil for food, feed, and industrial applications. However, soybean productivity is frequently constrained by drought stress [1, 2]. Under drought, limited water availability cause water deficit in plants, and soybean yield can be reduced by up to 40% [3, 4]. The frequency and severity of drought are expected to increase under climate change [5, 6]. Therefore, improving drought tolerance has long been a major objective in soybean breeding programs [7].

Drought tolerance involves coordinated physiological responses that maintain plant water status and growth under water-deficit conditions. As soil water availability decreases, root water uptake becomes insufficient to compensate for transpirational water loss, reducing tissue water status and cell turgor and causing leaf wilting. Water delivery to expanding tissues and nutrient uptake are also restricted, reducing cell expansion and plant growth [8]. To limit further water loss, drought-responsive signaling pathways promote stomatal closure [9]. Although stomatal closure helps maintain plant water status by reducing

transpiration, it also limits CO₂ availability and carbon assimilation, restricting plant growth and disrupting photosynthetic electron transport [10, 11].

Reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), are important regulators of physiological responses to water deficit [12]. Under water-deficit conditions, plasma membrane NADPH oxidases are activated in guard cells, generating ROS that mediate ABA-induced stomatal closure [13, 14]. However, reduced CO₂ assimilation following stomatal closure can disrupt photosynthetic electron transport and further increase ROS production under drought stress [10]. Because ROS function as signaling molecules but damage proteins, lipids, and nucleic acids when excessively accumulated, their production and scavenging must be balanced [13]. H₂O₂ accumulation reflects this balance and is used as a quantitative indicator of drought-induced oxidative stress [15, 16].

Previous genetic studies have identified numerous quantitative trait loci (QTLs) and candidate genes associated with drought tolerance in soybean [17–23]. Most studies have focused on agronomic traits, including yield, biomass, dry weight, or on physiological traits such as canopy wilting, and leaf water status. However, despite the importance of oxidative stress in drought responses, relatively few studies have investigated the genetic basis of ROS accumulation under drought conditions [24]. Consequently, it remains unclear how ROS accumulation is related to plant water status under drought stress and whether these physiological responses are governed by shared or distinct genetic factors.

To address these questions, three drought-response traits, wilting score, leaf moisture content (LMC), and leaf H₂O₂ content, were evaluated in 413 accessions of a Korean soybean core collection [25]. Phenotypic relationships among the three traits were examined, and genome-wide association studies (GWAS) based on whole-genome sequence variants were performed to identify and compare the genomic regions associated with each trait. By integrating phenotypic and genomic analyses, this study aimed to characterize the relationships between plant water status and ROS accumulation at both the phenotypic and genetic levels under drought stress.

Methods

Plant materials

A total of 413 accessions from the Korean soybean core collection was used in this study [25]. Whole-genome sequencing data for the accessions were obtained from the Soybean Haplotype Map Project [26]. Raw paired-end sequencing reads were processed using fastp (v0.24.0) with default parameters for adapter trimming and quality filtering [27]. Reads containing more than 40% low-quality bases, more than five ambiguous bases (N), or shorter than 15 bp after trimming were discarded. The filtered reads were aligned to the soybean reference genome Wm82.a6.v1 using BWA-MEM (v0.7.19) with default parameters, including a minimum seed length of 19bp, a mismatch penalty of 4, and gap-open and gap-extension penalties of 6 and 1, respectively [28, 29]. The resulting alignments were sorted and indexed using SAMtools (v1.22) [30].

Variant calling was performed following the Genome Analysis Toolkit (GATK, v4.6.2.0) workflow [31]. Briefly, genomic variants were called for each accession using HaplotypeCaller in GVCF mode, followed by joint genotyping using GenomicsDBImport and GenotypeGVCFs. Individual variant files were subsequently merged using GatherVcfs to generate the final VCF dataset.

Experimental design

All experiments were conducted in a greenhouse and were independently repeated three times. Soybean plants were grown in plastic pots (14 cm in diameter and 12 cm in height) containing 360g of commercial horticultural soil (Baroker, Seoul Bio Co., Korea). Within each replicate, pots were arranged randomly. To minimize border effects, pots containing the reference cultivar Williams 82 were placed around the perimeter of the experimental layout.

Drought stress treatment was initiated when the majority of plants had reached the V2 developmental stage, corresponding to 17, 17, and 16 days after sowing (DAS) in the experimental replicates. At the onset of drought treatment, each pot was covered with a plastic bag to minimize soil surface evaporation.

Phenotypic evaluation

Wilting score was visually evaluated using a five-point scale ranging from fully turgid leaves to complete leaf desiccation, according to the criteria described in a previous study [22]. Phenotypic evaluation and sampling were conducted when the largest proportion of plants within each experimental replicate exhibited a wilting score of 2, corresponding to 28, 30, 32 DAS in the replicates, respectively. LMC was determined using the second trifoliate leaf. Fresh weight (FW) was measured immediately after sampling. Leaf samples were then frozen in liquid nitrogen and stored at -80°C . Samples were subsequently freeze-dried for 72h, after which dry weight (DW) was measured. LMC was calculated as: $\text{LMC} = (\text{FW} - \text{DW}) / \text{FW}$. Leaf H_2O_2 content was determined using the third trifoliate leaf. Leaf samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. 0.1g of finely ground leaf powder was used for H_2O_2 quantification using the Amplex™ Red Hydrogen Peroxide/Peroxidase Assay Kit (Thermo Fisher Scientific) according to the manufacturer's instructions by measuring absorbance at 560nm. A hydrogen peroxide solution (Sigma-Aldrich) was used to generate a standard curve. H_2O_2 values below zero after standard-curve-based quantification were considered below the detectable range and were therefore set to zero.

To account for variation among experimental replicates, best linear unbiased predictions (BLUPs) were estimated using the lme4 package in R (v4.5.0), with environmental replicate fitted as a fixed effect and genotype as a random effect [32]. The resulting BLUP values were used for subsequent GWAS analyses.

Genome wide association studies

For GWAS, only high-quality biallelic SNPs were retained after filtering for a missing genotype rate $\leq 10\%$, a minor allele frequency (MAF) $\geq 5\%$, and a heterozygosity rate $\leq 10\%$. The LMC phenotypic values were transformed using the Box-Cox transformation prior to GWAS analysis [33].

Genome-wide association analyses were performed using the GAPIT3 package with four statistical models: MLM, MLMM, FarmCPU, BLINK [34]. The significance threshold for marker-trait associations was determined using the Bonferroni correction at a genome-wide significance level of $P = 0.05/N$, where N represents the total number of SNPs analyzed [35].

Search of putative candidate genes

Putative candidate genes were identified within the linkage disequilibrium (LD) blocks surrounding the lead SNPs of each significant association locus. LD blocks were defined using the default parameters implemented in LDblockShow (v1.41.) [36]. Candidate genes were prioritized based on previously reported associations with drought stress in soybean or homologs in *Arabidopsis thaliana*. Functional annotations of sequence variants within Candidate genes regions were predicted using SnpEff (v5.2) [37]. Gene annotations and functional information were obtained from the GlycineMine database from LIS (<https://mines.legumeinfo.org/glycinemine/>).

Syntenic regions surrounding *Glyma.13G183500* were identified using MCScanX with default parameters, based on BLASTp comparisons of the soybean proteome [38]. Comparative genomic analysis was performed using a ± 500 kb genomic region centered on *Glyma.13G183500* (Gm13:29,470,642 – 30,490,537 bp).

Results

Phenotypic variation in drought response traits of the soybean core collection

To characterize variation in drought responses in a Korean soybean core collection, we evaluated three physiological traits under controlled drought conditions: wilting score, LMC, and leaf H_2O_2 content. All three drought-response traits exhibited continuous phenotypic variation across the soybean core collection (Fig. 1a).

Wilting scores were distributed across the five-point rating scale, indicating broad variation in visual drought symptoms among accessions. The mean wilting scores were 2.93, 2.73, and 2.87 across the three environmental replicates, with corresponding standard deviations (SDs) of 1.29, 1.11, and 1.15, respectively. LMC ranged from 0.06–0.89, 0.07–0.92, and 0.00–0.94 across the three replicates, with mean values of 0.69, 0.74, and 0.75 and corresponding SDs of 0.21, 0.18, and 0.21, respectively. H_2O_2 content ranged from 0–75.76, 0–70.74, and 0–88.20 $\mu\text{g/g}$ across the three replicates, with mean values of

23.18, 26.10, and 33.68 µg/g and corresponding SDs of 17.30, 20.55, and 19.58 µg/g, respectively (Supplementary Table S1).

The coefficient of variation (CV) differed among the three traits, ranging from 39.9–44.1% for wilting score, 25.1–30.2% for LMC, and 58.1–78.7% for H₂O₂ content (Supplementary Table S1). Broad-sense heritability (H^2) was 0.56 for wilting score, 0.47 for LMC, and 0.59 for H₂O₂ content, indicating moderate heritability across all three traits and suggesting a substantial genetic contribution to phenotypic variation and potential for genetic improvement.

Correlation analysis revealed a strong negative relationship between wilting score and LMC ($r = -0.79$, $P < 0.001$), indicating that the visual wilting score reliably reflects leaf water status (Fig. 1b). In contrast, H₂O₂ content showed significant but weak correlations with both wilting score ($r = -0.20$, $P < 0.001$) and LMC ($r = 0.30$, $P < 0.001$), suggesting that H₂O₂ accumulation may represent a physiological component of the drought response that is distinct from changes in plant water status and wilting symptoms.

Genome wide association analysis of wilting score and LMC in the soybean core collection

After quality filtering, a total of 4,942,357 high-quality SNPs were retained and used for subsequent GWAS analyses. Genome-wide association analysis identified two significant loci for wilting score and five for LMC (Fig. 2a-b). Among these, a locus on chromosome 10 was the only genomic region significantly associated with both traits. This locus was the only association consistently detected by all four GWAS models (MLM, MLMM, FarmCPU, BLINK) for both wilting score and LMC, providing strong evidence for a shared genetic component of leaf water content and wilting at this locus. Furthermore, this genomic region co-localized with a previously reported drought-associated QTL identified using the same phenotyping protocol, further supporting the association of this locus with drought response [22].

To further refine the associated region, linkage disequilibrium (LD) block analysis was performed. The LD block encompassing the chromosome 10 locus contained 20 annotated genes. Among these, *Glyma.10G019000* was the only gene with previous evidence of involvement in drought stress based on functional annotation of the gene itself, its paralogs, and homologs in *Arabidopsis thaliana* [39, 40]. *Glyma.10G019000*, which encodes an ATP-binding cassette (ABC) transporter, was therefore selected as the primary candidate gene for subsequent haplotype analysis.

Haplotype analysis identified three major haplotypes defined by 6 SNPs within *Glyma.10G019000* (Fig. 2c). Of the six SNPs, one was located in the 5' UTR, one in the coding region, and four in the 3' UTR. The coding-region SNP was a missense variant predicted to alter the amino acid sequence of the encoded protein. Comparison of phenotypic differences among haplotypes revealed that Hap1 differed significantly from Hap2 and Hap3 for wilting score. For LMC, all three haplotypes exhibited significant pairwise differences. These results demonstrate a significant association between allelic variation in *Glyma.10G019000* and both wilting score and LMC under drought stress.

Genome wide association analysis of H₂O₂ content in the soybean core collection

GWAS for H₂O₂ content identified a total of eight significant QTLs (Fig. 3a). None of these loci overlapped with the QTLs associated with wilting score or LMC, suggesting that H₂O₂ accumulation is governed by genetic factors largely distinct from those underlying dehydration-related traits. Among the eight QTLs, a single locus on chromosome 13 was consistently detected by all four GWAS models (Table 1).

LD block analysis of the chromosome 13 locus identified a 19.9-kb LD block containing a single annotated gene, *Glyma.13G183500*, within the associated interval (Fig. 3b). Although this gene has not previously been reported to be associated with drought stress, it encodes a polyphenol oxidase, an enzyme involved in the oxidation of polyphenolic compounds and potentially associated with cellular redox regulation. Based on its location within the associated LD block and its potential role in redox regulation, *Glyma.13G183500* was considered a promising candidate gene for the chromosome 13 locus and was subsequently subjected to haplotype analysis.

Haplotype analysis identified six major haplotypes defined by ten SNPs within the promoter region of *Glyma.13G183500* (Fig. 3c). Hap1 and Hap2 exhibited relatively low H₂O₂ accumulation, whereas Hap3, Hap5, and Hap6 showed relatively high H₂O₂ levels. These results indicate that allelic variation in *Glyma.13G183500* is associated with natural variation in oxidative stress responses under drought conditions.

Several previously reported GWAS signals associated with drought and other abiotic stress-related traits were located in the genomic region surrounding *Glyma.13G183500*, including signals for canopy wilting, plant weight under drought, and cold imbibition stress (Fig. 3d) [19, 41–44]. In addition, syntenic regions on Gm07, Gm13 (35.105–35.687 Mb), and Gm15 also harbored previously reported GWAS signals for stress-related traits, including canopy wilting, germination index under drought, response to off-target dicamba, and seedling length under flooding [19, 45–47]. These observations further highlight the potential relevance of this genomic region to abiotic stress responses.

Discussion

Drought stress is a major challenge to soybean productivity, particularly under increasingly variable water availability associated with climate change [1, 48]. Drought tolerance is governed by multiple physiological processes that collectively determine plant performance under water-deficit conditions [7, 8]. Understanding the phenotypic and genetic relationships among these processes is important for improving drought tolerance in soybean. Because these processes represent different aspects of the drought response, their genetic determinants may not necessarily coincide. Understanding how these physiological components are genetically coordinated is important for developing soybean cultivars with more effective drought-response strategies. In the present study, wilting score and LMC were strongly

correlated, and both traits were associated with the same locus on chromosome 10. H₂O₂ content showed weak correlations with wilting score and LMC, and was associated with a different locus on chromosome 13. These results suggest that H₂O₂ accumulation may reflect an aspect of the drought response that differs from LMC and wilting at both the phenotypic and genetic levels.

The qWS_10_1 and qLMC_10_1 identified in this study were located within the interval of qSW_Gm10 a stable drought-response QTL previously identified using wilting score and LMC across multiple years [22]. The consistent detection of this region in independent populations provides further evidence for its association with plant water status under drought stress. *Glyma.10G019000*, identified as a candidate gene in this study, encodes an ATP-binding cassette (ABC) transporter of the ABCC subfamily, also known as a multidrug resistance-associated proteins (MRPs). ABCC transporters are predominantly localized to the tonoplast, where they mediate the vacuolar sequestration of toxic compounds and secondary metabolites [49]. In *Arabidopsis thaliana*, the ABCC transporters AtABCC1 and AtABCC2 have been shown to transport abscisic acid glucosyl ester (ABA-GE), contributing to ABA storage and remobilization [50]. ABCC-mediated ABA transport has also been implicated in ABA-dependent stomatal regulation [51]. These findings suggest that *Glyma.10G019000* may contribute to drought tolerance by modulating ABA homeostasis and stomatal responses, which could help maintain plant water status under drought stress.

The co-occurrence of previously reported GWAS signals for various abiotic stresses including drought stress within or near the chromosome 13 locus suggests that this region may influence multiple aspects of plant stress responses (Fig. 3d). These previously reported associations encompass various aspects of stress response, ranging from drought-related traits such as canopy wilting and plant growth under water deficit to responses to cold, flooding, and herbicide exposure. Rather than being limited to a single physiological process, the range of traits associated with this region may reflect the complex nature of plant responses to drought and other abiotic stresses. ROS production and scavenging are involved in plant responses to multiple abiotic stresses [13]. Although the primary effects of these stresses differ, disruption of cellular redox homeostasis is a common feature of many abiotic stress responses. Our study extends these previous findings by directly quantifying H₂O₂ accumulation and identifying genetic variation associated with this oxidative response at the chromosome 13 locus. The use of H₂O₂ accumulation as a physiological trait provides an additional layer for dissecting stress responses beyond visible or growth-related phenotypes, highlighting ROS homeostasis as an important component linking genetic variation in this region with diverse responses to drought and other abiotic stresses.

Glyma.13G183500, identified as a candidate gene for the chromosome 13 locus associated with H₂O₂ accumulation, encodes a polyphenol oxidase (PPO). In plants, PPOs have been implicated in responses to water deficiency in several species. In wild soybean (*Glycine soja*), drought stress increased PPO expression at both the transcript and protein levels, indicating that PPO may be involved in drought adaptation [52]. A recent functional study further demonstrated that *PmPPO* from *Prunus mira* was induced by drought and ABA, and that its overexpression enhanced drought tolerance, increased ROS-scavenging capacity, and reduced oxidative damage [53]. These findings suggest that PPOs may

participate in drought adaptation through ROS regulation and redox homeostasis. The association of *Glyma.13G183500* with H₂O₂ accumulation in our study is consistent with the reported involvement of PPOs in ROS regulation under drought stress. In addition, this genomic region exhibited an XP-CLR signal between improved cultivars and landraces, suggesting that it may have been subjected to artificial selection during soybean improvement [54]. Given the association of the region with H₂O₂ accumulation and diverse abiotic stress-related traits, genetic variation at this locus may have been unintentionally subjected to selection during breeding for adaptation and agronomic performance under various environmental conditions.

Although H₂O₂ accumulation provided a quantitative measure of oxidative responses under drought, additional measurements of antioxidant activity and oxidative damage are needed to determine whether differences in H₂O₂ content are accompanied by broader changes in redox homeostasis. In addition, the functions of *Glyma.10G019000* and *Glyma.13G183500* in drought responses remain to be experimentally validated in soybean. Further integration of these complementary drought-response traits with functional validation of the identified candidate genes will help clarify how different physiological processes collectively determine plant performance under drought.

Conclusions

This study revealed that soybean drought responses comprise physiologically related but genetically differentiated components. Wilting score and LMC were strongly correlated and shared a major association locus on chromosome 10, whereas H₂O₂ accumulation was only weakly correlated with these traits and was associated with a distinct locus on chromosome 13. *Glyma.10G019000* and *Glyma.13G183500* were identified as candidate genes for the chromosome 10 and chromosome 13 loci, respectively. To our knowledge, this study provides the first evidence that genetic variation in *Glyma.13G183500* is associated with H₂O₂ accumulation under drought stress in soybean. These findings demonstrate the value of integrating phenotypes across different physiological levels, including whole-plant wilting, tissue water status, and cellular oxidative responses, to improve our understanding of the genetic architecture of drought responses and facilitate the identification of genetic components that may not be captured by a single phenotype.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

M.C. and J.Y. conceptualized the study, developed the methodology, conducted the experiments, performed the formal analyses, curated the data, prepared the visualizations, and contributed to writing the manuscript. H.K. contributed to the conceptualization and methodology of the study and to writing the manuscript. Y.C. contributed to the methodology, conducted experiments, and curated the data. C.K. and G.A. conducted experiments and contributed to data curation. SY.S. provided resources for the study. J.H. contributed to the conceptualization and methodology of the study, supervised the research, and acquired funding. All authors read and approved the final manuscript.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

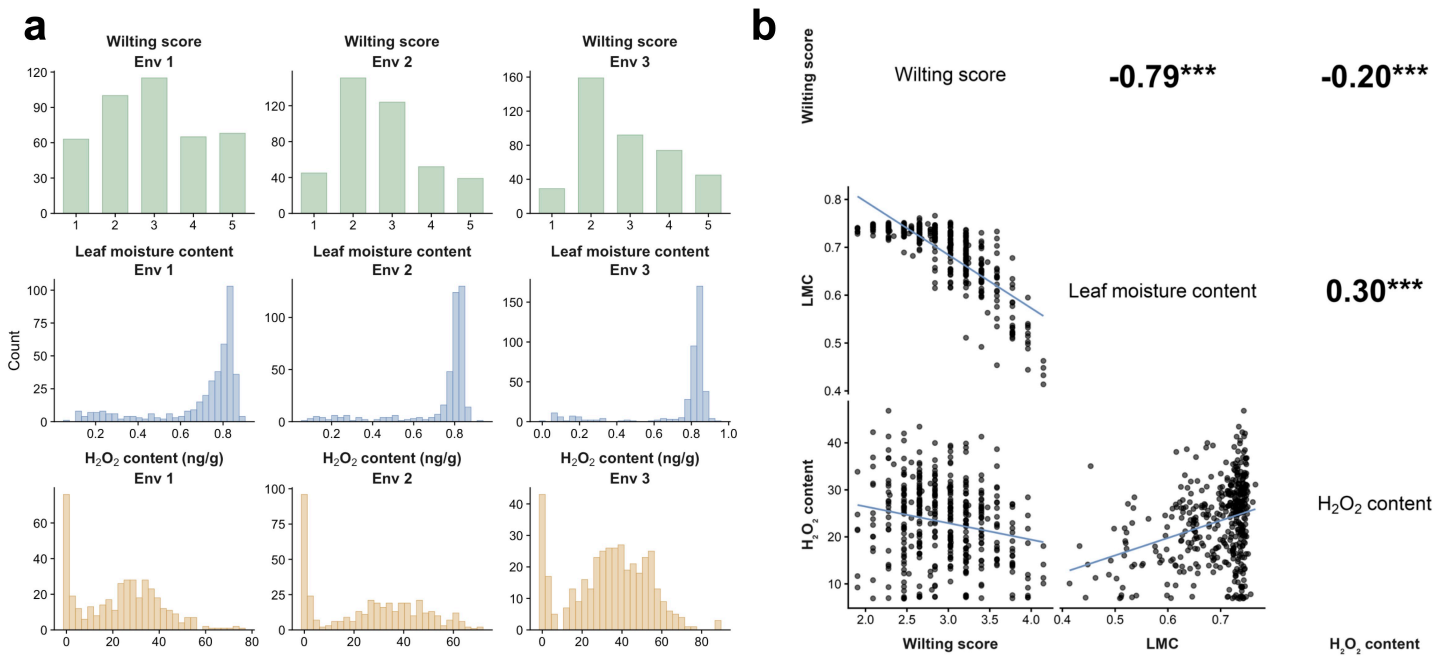


Figure 1

Phenotypic variation and relationships among drought stress-related traits in the soybean core collection. (a) Distribution of wilting score, LMC, and H₂O₂ content across three experimental replicates under drought stress. (b) Pairwise scatter plots and Pearson correlation coefficients among wilting score, LMC, and H₂O₂ content. Scatter plots are shown in the lower panels and Pearson correlation coefficients in the upper panels. Blue lines indicate linear regression fits. Statistical significance is indicated as * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

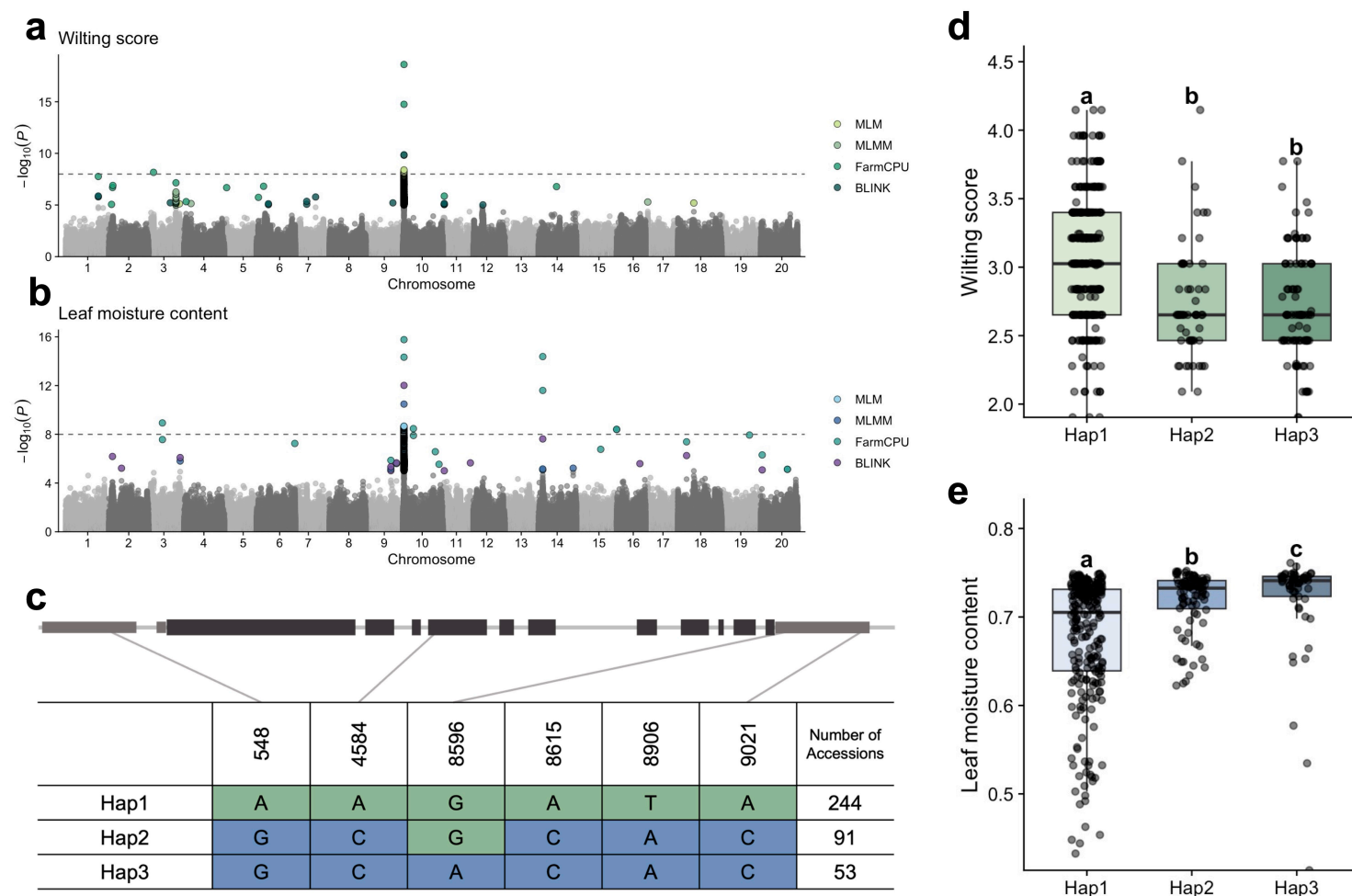


Figure 2

Identification of a shared genomic region associated with wilting score and LMC. (a–b) Manhattan plots of genome-wide association analyses for (a) wilting score and (b) LMC. Results obtained using four GWAS models (MLM, MLMM, FarmCPU, and BLINK) are overlaid. The horizontal dashed line indicates the Bonferroni-corrected significance threshold ($P = 1.01166 \times 10^{-8}$). (c) Haplotype structure of *Glyma.10G019000*. Numbers above each polymorphic site indicate its position relative to the coding sequence (CDS) start site. The rightmost column indicates the number of accessions assigned to each haplotype. (d–e) Phenotypic differences among *Glyma.10G019000* haplotypes for (d) wilting score and (e) LMC. Different letters indicate significant differences among haplotypes based on pairwise Wilcoxon tests with Benjamini–Hochberg correction ($P < 0.05$).

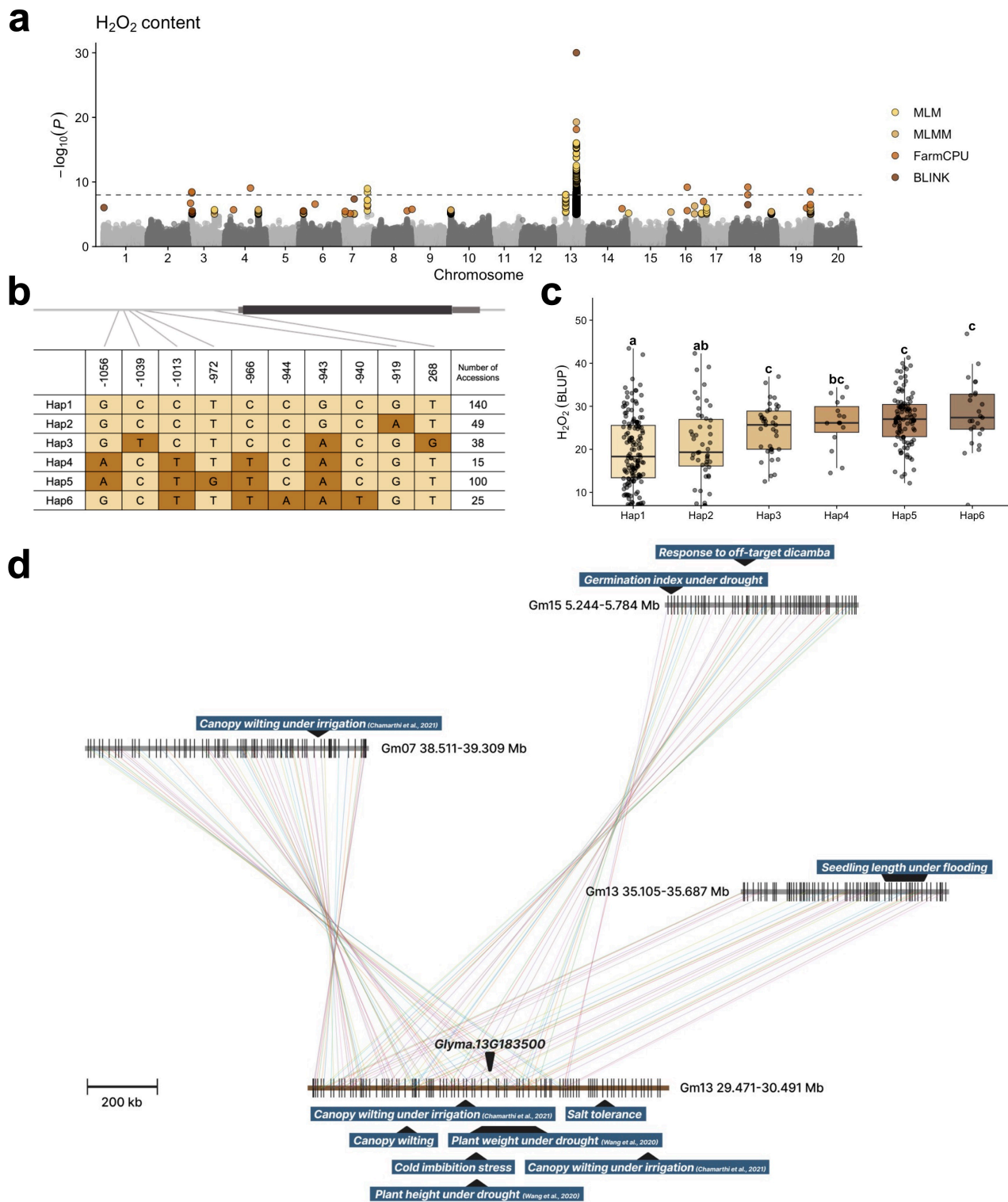


Figure 3

Identification of a genomic region associated with H_2O_2 content under drought stress. (a) Manhattan plot of genome-wide association analyses for H_2O_2 content. Results obtained using four GWAS models (MLM, MLMM, FarmCPU, and BLINK) are overlaid. The horizontal dashed line indicates the Bonferroni-corrected significance threshold ($P = 1.01166 \times 10^{-8}$). (b) Haplotype structure of *Glyma.13G183500*. Numbers above each polymorphic site indicate its position relative to the coding sequence (CDS) start

site. The rightmost column indicates the number of accessions assigned to each haplotype. (c) Phenotypic differences in H₂O₂ content among *Glyma.13G183500* haplotypes. Different letters indicate significant differences among haplotypes based on pairwise Wilcoxon tests with Benjamini–Hochberg correction ($P < 0.05$). (d) Syntenic relationships between the genomic region containing *Glyma.13G183500* on chromosome 13 and homologous regions on chromosome 7, 13, and 15. Colored lines connect syntenic gene pairs among the corresponding genomic regions. Previously reported QTLs associated with abiotic stress related traits, are indicated along each region. *Glyma.13G183500* is marked by a black arrowhead.

Supplementary Files

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